DOI:
10.1016/j.jinf.2018.01.001

Document Version
Peer reviewed version

Accepted Manuscript

Title: Potential of real-time PCR threshold cycle (CT) to predict presence of free toxin and clinically relevant c. difficile infection (CDI) in patients with cancer: a reply

Author: Stephanie Wilmore, Simon D. Goldenberg

PII: S0163-4453(18)30001-X
DOI: https://doi.org/10.1016/j.jinf.2018.01.001
Reference: YJINF 4038

To appear in: *Journal of Infection*

Accepted date: 6-1-2018

Please cite this article as: Stephanie Wilmore, Simon D. Goldenberg, Potential of real-time PCR threshold cycle (CT) to predict presence of free toxin and clinically relevant c. difficile infection (CDI) in patients with cancer: a reply, *Journal of Infection* (2018), https://doi.org/10.1016/j.jinf.2018.01.001.

COMMENT LETTER

Potential of real-time PCR threshold cycle (CT) to predict presence of free toxin and clinically relevant C. difficile infection (CDI) in patients with cancer: A reply

Stephanie Wilmore and Simon D. Goldenberg*

Centre for Clinical Infection and Diagnostics Research (CIDR), King's College, London and Guy's & St Thomas' NHS Foundation Trust, London UK.

*Corresponding author

Westminster Bridge Road, London SE1 7EH

Simon.goldenberg@gstt.nhs.uk

0207 188 8515

To the Editor,

The recent manuscript by Kamboj and colleagues highlights an interesting potential use of molecular assays in the diagnosis of *Clostridium difficile* infection (CDI). (1)

There are a number of other recent articles that have shown that PCR cycle threshold (CT) values may have some utilty in predicting severe infection (2) or have previously been used as a surrogate marker for bacterial load and disease activity when assessing differences between patient groups such as potential *C. difficile* excretors compared with those with infection. (3-5)

Similarly, our institution has also observed a relationship between PCR CT values and toxin EIA positivity. Between February 2011 and August 2017 a total of 1402 diarrhoeal stool samples were tested and found to be positive for presence of toxigenic *C. difficile* using our standard laboratory algorithm. This consists of a GDH (glutamate dehydrogenase) immunoassay (GDH Chek™-60, TechLab, Blacksburg, VA), followed by a toxin A/B EIA (C. difficile Tox A/B II™, Techlab, Blacksburg, VA) and PCR (GeneXpert, Cepheid, Sunnyvale, CA). A report is issued as 'toxigenic *C. difficile* detected' if either toxin A/B is detected OR if the PCR is positive.

A total of 833 samples were PCR positive but toxin EIA negative and a total of 569 samples were both PCR and Toxin EIA positive. Median PCR CT values were significantly lower in samples that were toxin EIA positive (23.9 IQR, 22, 26.2) compared with samples that were toxin EIA negative (29.2 IQR, 19.1, 32.2, $p<0.001$), suggesting greater organism load. Figure 1.

A ROC curve was generated using toxin EIA as the reference method and resulted in an AUC of 0.806 (95% Confidence interval 0.784-0.829), which is similar to the AUC figure of Kamboj et al (0.83). Figure 2. The AUC and maximum Youden index value (0.502) yielded an optimal CT value threshold of 27.0, slightly lower than that of Kamboj et al of 28.0. This resulted in a sensitivity and specificity of 83.1% (95% CI, 79.8-86.1%) and 67% (95% CI, 63.7-70.2%) and positive and negative predictive values of 63.3% (95% CI, 59.7-66.7%) and 85.3% (95% CI, 82.4-87.9%) respectively.

A total of 886 (63%) had accompanying ribotyping data, however there were no significant differences in the distribution of ribotypes in the toxin EIA positive and negative groups. Most

common ribotypes were; 014/020 (19.8%), 015 (12.6%), 002 (12.3%), 005 (8.0%), 078 (6.7%), 001 (3.6%). Ribotype 027 comprised only 1% (9 samples).

Although we have not collected clinical data on infection/excretor status of these patients, most of those who are toxin EIA positive do have CDI and are treated as such. However, there are a significant number of patients who are toxin EIA negative yet are still likely to have CDI and are managed as such (often with costly medication such as fidaxomicin). Furthermore, there is a significant overlap of Ct values in those that are toxin EIA positive and negative, making it difficult in practice to use such Ct values to definitively categorise patients in this way. Although the sensitivity of using a PCR with a calculated cut off CT value as a single test is high, the specificity of this approach is suboptimal and the significant risk of misclassifying patients (in incorrectly treating them) remains.

There is good evidence from at least three studies demonstrating that clinical outcomes, including CDI related complications and deaths are correlated with toxin EIA or cell cytotoxicity assay and that use of PCR alone is likely to lead to overdiagnosis of CDI, resulting in overtreatment and wasting resources. (6-8)

Cell cytotoxin neutralisation is both highly sensitive and specific, however it is laborious and slow, therefore results cannot be provided in a clinically relevant timeframe. It would also not be possible to categorise patients into excretors or infected, nor to predict risk of severe infection using this method. For these reasons it is routinely used by few centres. (9)

Classifying symptomatic patients into infected and excretor status may be important in terms of infection prevention and control, since excretors are just as likely to contaminate the clinical environment with spores as those with infection. (3)

As recommended by European guidelines (10) the use of GDH and toxin EIA is highly specific, resulting in a false positive rate of 0.4-0.6%, (7) however the estimated 95% confidence interval for sensitivity is 78.8-84.5%, resulting in a false negative rate of 15.5-21.2%.

Thus, although the use of PCR Ct values may be valuable in determining severity of infection, risk of recurrence and mortality, it is difficult to envisage that the use of a PCR alone would be sufficient test of true infection.

GDH (or PCR) followed by a toxin EIA remains the most optimal and practical method available today, however diagnostics manufacturers should be challenged to develop better performing tests which are rapid and both highly sensitive and specific.

References

1. Kamboj M, Brite J, McMillen T, Robilotti E, Herrera A, Sepkowitz K et al. Potential of real-time PCR threshold cycle (CT) to predict presence of free toxin and clinically relevant c. difficile infection (CDI) in patients with cancer. J Infect (in press)

2**. Senchyna F, Rajiv L. Gaur RL, Gombar S, Truong CY, Schroeder LF, Banaei N.** Clostridium difficile PCR cycle threshold predicts free toxin. J Clin Microbiol 2017; 55:2651-2660.

3. **Biswas JS, Patel A, Otter JA, van Kleef E, Goldenberg SD**. Contamination of the hospital environment from potential *Clostridium difficile* excretors without active infection. Infect Control Hosp Epidemiol 2015;**36**:975-7.

4. **Kamboj M, Babady NE, Marsh JW, Schlackman JL, Son C, Sun J, Eagan J, Tang YW, Sepkowitz K.** Estimating risk of *C. difficile* transmission from PCR positive but cytotoxin negative cases. PLoS One 2014;**9**:e88262.

5. **Dionne LL, Raymond F, Corbeil J, Longtin J, Gervais P, Longtin Y.** Correlation between *Clostridium difficile* bacterial load, commercial real-time PCR cycle thresholds, and results of diagnostic tests based on enzyme immunoassay and cell-culture cytotoxicity assay. J Clin Microbiol 2013;**51**:3624-30.

6. **Longtin Y, Trottier S, Brochu G, Paquet-Bolduc B, Garenc C, Loungnarath V, Beaulieu C, Goulet D, Longtin J.** Impact of the type of diagnostic assay on *Clostridium difficile* infection and complication rates in a mandatory reporting program. Clin Infect Dis 2013;**56**:67-73.

7. **Planche TD, Davies KA, Coen PG, Finney JM, Monahan IM, Morris KA, O'Connor L, Oakley SJ, Pope CF, Wren MW, Shetty NP, Crook DW, Wilcox MH.** Differences in outcome according to *Clostridium difficile* testing method: A prospective multicentre diagnostic validation study of *C difficile* infection. Lancet Infect Dis 2013;**13**:936-45.

8. **Polage CR, Gyorke CE, Kennedy MA, Leslie JL, Chin DL, Wang S, Nguyen HH, Huang B, Tang YW, Lee LW, Kim K, Taylor S, Romano PS, Panacek EA, Goodell PB, Solnick JV, Cohen SH**. Overdiagnosis of *Clostridium difficile* infection in the molecular test era. JAMA Intern Med 2015;**175**:1792-801.

9. **Goldenberg SD, French GL**. Diagnostic testing for *Clostridium difficile*: A comprehensive survey of laboratories in England. J Hosp Infect 2011;**79**:4-7.

10. **Crobach MJ, Planche T, Eckert C, Barbut F, Terveer EM, Dekkers OM, Wilcox MH, Kuijper EJ.**. European Society of Clinical Microbiology and Infectious Diseases: Update of the diagnostic guidance document for *Clostridium difficile* infection. Clin Microbiol Infect 2016;**22**:S63-81.

Figure 1

Comparison of *Clostridium difficile* PCR cycle threshold values of stool samples from patients who also tested toxin A/B EIA negative (n=833) and positive (n=569). The horizontal broken line indicates the median; whereas the top and bottom solid lines represent the 75^{th} and 25^{th} centiles, respectively.

Figure 2

Receiver-operating curve (ROC) of *tcdB* cycle threshold (CT) values for detection of free toxin by enzyme immunoassay.